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The side effects of the herbicides atrazine and paraquat upon *Folsomia candida* and *Tullbergia granulata* (Insecta, Collembola)

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With 6 figures

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1. Introduction

Field studies have shown that some herbicides decrease Collembola populations, while others increase them or have no apparent effects (RAPOPORT & CANGLIOLI 1963; FOX 1964). CURRY (1970) reported that application of paraquat in grassland decreased populations of Collembola and Acari, but EDWARDS et al. (1972) found more arthropods and fewer Acari in treated plots. Earlier, EDWARDS (1970) observed that paraquat had no apparent effect on small invertebrates. Studies on the effects of triazines, however, yielded consistent results. This group of herbicides was found to decrease Collembola populations as well as other soil arthropods, and the effects may persist for longer periods (FOX 1964; EDWARDS 1970; POPOVICI et al., 1977). A review of the effects of herbicides on Collembola as well as on other soil animals is found in EDWARDS & THOMPSON (1973).

Laboratory studies can provide valuable information on the effects of herbicides upon soil animals. This has been shown clearly by EIJSACKERS (1975; 1978a, b) in his studies on the effects of the herbicide 2,4,5-T on *Onychiurus quadriocellatus* GISIN. The present study deals with herbicide effects on the biology of Collembola, *Folsomia candida* (WILLEM) and *Tullbergia granulata* MILLS, and is viewed as a basis for future investigations.

2. Material and methods

In this investigation, commercial formulations of atrazine and paraquat were used. Both are now being applied in till/no-till corn fields on the Soil Research Plots at Michigan State University. Two species of Collembola, *Folsomia candida* (WILLEM) and *Tullbergia granulata* MILLS, were selected as test animals. *F. candida* is a common laboratory species which is widely used in soil arthropod experiments, while *T. granulata* was collected from an old field at the Soil Research Plots. Both species are parthenogenetic and all test individuals were females.

Laboratory rearing techniques basically followed methods described by SNIDER et al. (1969), utilizing small plastic jars 27 mm high and 34 mm in diameter. A mixture of plaster-charcoal in an 8:5 ratio was prepared for the rearing substrate. Cultures were maintained at approximately 100 percent relative humidity by dropping distilled water on the substrate daily or as required. Cultures were kept in a controlled temperature incubator at 15.5 °C (60 °F) in constant darkness.

Eggs of both species were transferred from stock cultures to experimental jars with a needle, one egg per jar. Starting on the day of hatching, the Collembola were fed brewer's yeast treated with herbicide. The contaminated food was renewed every four days or as required, to prevent it from being invaded by fungal mycelium.

Treated yeast was prepared as follows: 350 grams powdered brewer's yeast was suspended in 100 ml of a desired concentration of herbicide. The suspension was then filtered, and the filtered yeast was dried at room temperature. Because the solubility of atrazine is limited, crystals formed on the filter when dried. Crystals and dried yeast were then mixed together and used as food. The experiment was run with three concentrations of each herbicide (paraquat and atrazine): 600 ppm, 1,000 ppm, and 5,000 ppm a.i. Concentrations applied to the field are about 600 ppm for paraquat, and approximately 5,000 ppm for atrazine. These values were calculated from field applications suggested by the Cooperative Extension Service, Michigan State University (CHASE & MEGGIT 1976; NELSON et al., 1976).

Observations were conducted daily and terminated after 22 weeks. Table 1 summarizes the experimental design.

Table 1. Number of treatment replicates*

Species	Control	Paraquat			Atrazine		
		600 ppm	1,000 ppm	5,000 ppm	600 ppm	1,000 ppm	5,000 ppm
F. candida	20	20	20	20	20	20	20
T. granulata	20	20	20	20	20	20	20

* One individual per replicate container.

Table 2. *Folsomia candida*, cumulative percent mortality, at 15.5 °C (60 °F)

Week	Control	Paraquat			Atrazine		
		600 ppm	1,000 ppm	5,000 ppm	600 ppm	1,000 ppm	5,000 ppm
6	0	0	0	0	0	0	5
9	0	0	0	0	0	10	10
11	0	0	0	0	0	10	10
12	0	0	0	0	0	10	15
15	5	0	0	0	0	10	30
18	5	0	5	0	0	10	30
21	5	0	10	5	5	10	30
22	5	0	10	5	5	10	30

Table 3. *Tullbergia granulata*, cumulative percent mortality, at 15.5 °C (60 °F)

Week	Control	Paraquat			Atrazine		
		600 ppm	1,000 ppm	5,000 ppm	600 ppm	1,000 ppm	5,000 ppm
2	0	0	0	0	0	0	15
3	0	0	0	0	0	0	35
4	0	0	0	0	0	0	50
5	0	0	0	0	0	0	55
22	0	0	0	5	0	0	55

3. Results

3.1. Mortality

Only one individual in the control *F. candida* died. Three individuals of *F. candida* fed on paraquat died (2 at 1,000 ppm and one at 5,000 ppm). Similar mortalities occurred with atrazine at 600 ppm and 1,000 ppm. However, six individuals or 30 percent of *F. candida* fed on 5,000 ppm atrazine died during the 22-week period (Table 2).

T. granulata experienced mortality at only 5,000 ppm of paraquat and atrazine. At 5,000 ppm of paraquat, one individual died, as opposed to eleven individuals at 5,000 ppm of atrazine. As in *F. candida*, death occurred in the early weeks, and those that did not die survived until the end of the experiment (22 weeks; Table 3). At 5,000 ppm of atrazine, both species were found to be repelled by the food and were rarely seen feeding. This did not occur at lower concentrations of atrazine, nor at any concentration of paraquat.

3.2. Instar duration

Data presented by SNIDER (1971) suggest that instar duration can be determined by the presence of exuvia. Exuvia were therefore recorded and then removed. *F. candida* tend to eat their own exuvia (SNIDER 1971), but daily observation made it possible to find exuvial

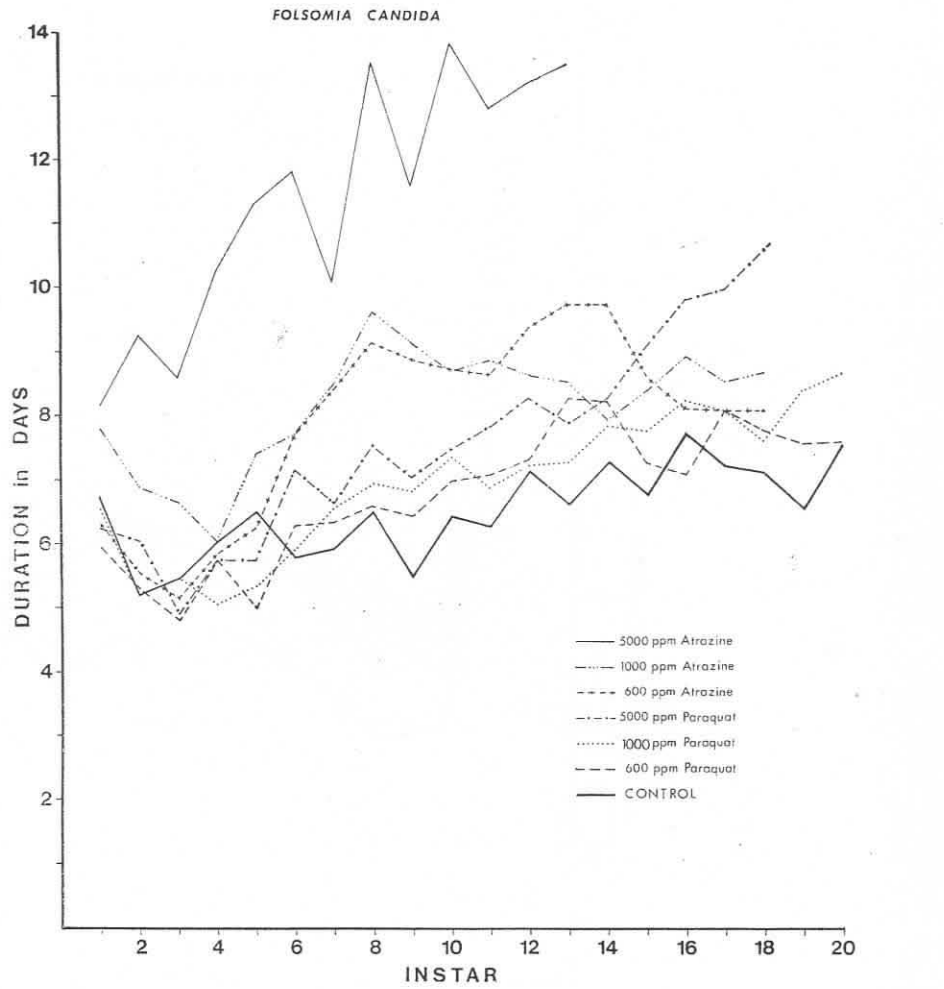

Fig. 1. *Folsomia candida*, instar duration at 15.5 °C (60 °F).

remains. However, *F. candida* fed on atrazine, especially at 5,000 ppm, ate their exuvia soon after ecdysis. Although the very small remaining portions may have been overlooked, they usually could be located after a careful search. *T. granulata* does not eat its own exuvia, so that observational difficulties arose only in determining ecdyses of young instars because these Collembola are very small.

Mean instar durations of *F. candida* and *T. granulata* are illustrated in Figures 1 and 2. In general, beginning at the sixth instar, treated *F. candida* had a longer instar duration than controls. Significant extension of duration occurred in individuals fed on 5,000 ppm of paraquat and at all concentrations of atrazine. Compared to lower concentrations by Tukey's t-test (TUKEY 1953), 5,000 ppm of atrazine significantly increased instar duration ($p \leq .05$).

In *T. granulata*, all treated animals exhibited a longer instar duration than controls (Figure 2), differences being significant for every stadium (Tukey's t-test, $p \leq .05$). As in *F. candida*, instar duration of *T. granulata* fed on 5,000 ppm of atrazine was significantly longer when compared to lower concentrations.

Aside from high mortality in both species at 5,000 ppm of atrazine, not all survivors reached the same instar. Only six individuals of *F. candida* out of fourteen surviving the

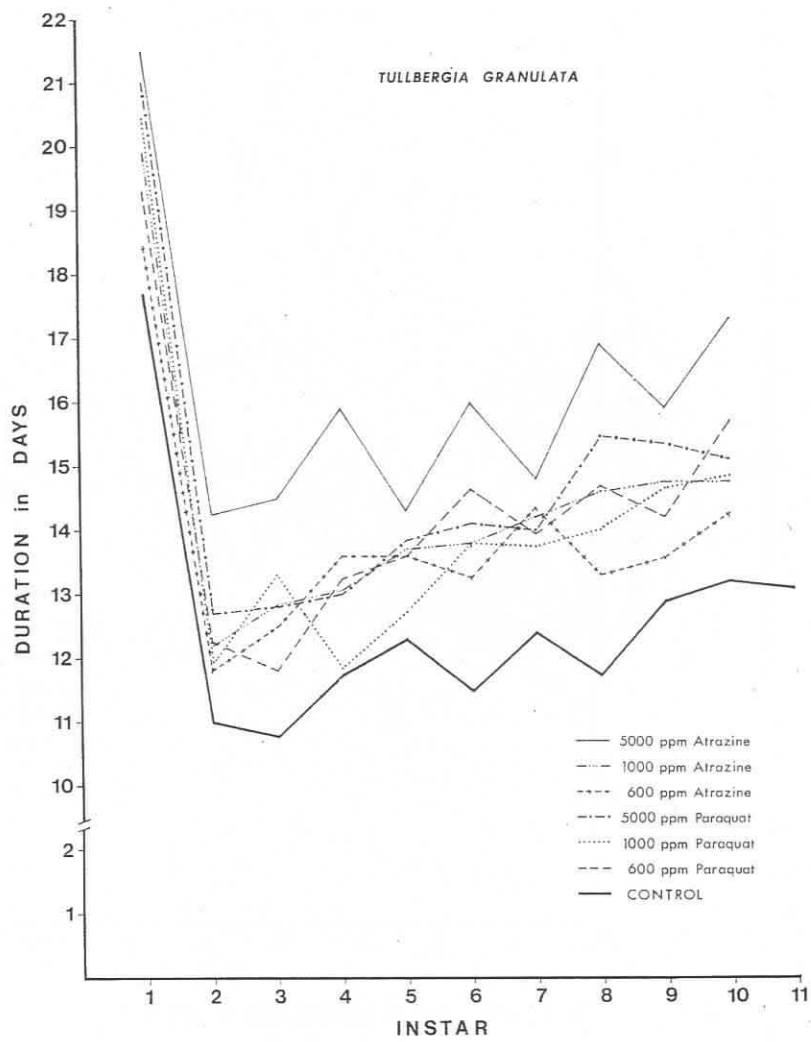

Fig. 2. *Tullbergia granulata*, instar duration at 15.5 °C (60 °F).

22-week period had reached the thirteenth instar. In *T. granulata*, among eleven individuals surviving, only ten had reached the seventh instar; nine reached eighth; four reached ninth; and three reached tenth. All the control animals reached the same instar; *F. candida* reached the twentieth and *T. granulata* reached the tenth instar. Obviously treated animals reached a lower stadium because of longer duration. Both species at 5,000 ppm of atrazine were considerably smaller than controls at the same instar. Although no measurements were taken during the experiment, size differences were obvious.

3.3. Egg production

F. candida laid eggs shortly after the fifth ecdysis was completed and then, more or less regularly, in every other stadium (SNIDER 1971). In this experiment, almost all the controls laid eggs regularly in every other stadium, beginning in the sixth. Only one individual was found to lay eggs at the seventeenth, nineteenth and twenty-first instead of at the sixteenth, eighteenth, and twentieth instar. In treated *F. candida*, irregularities in ovipositions were commonly found.

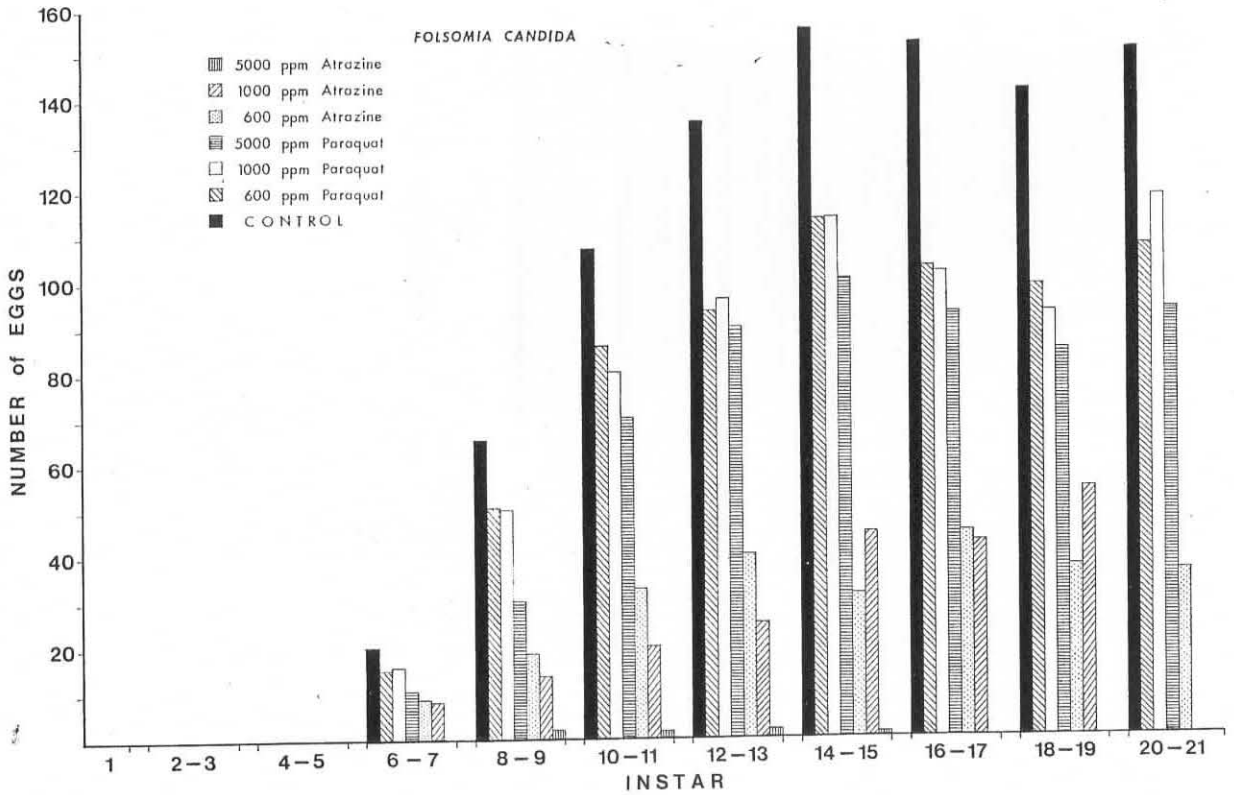

Fig. 3. *Folsomia candida*, egg production at 15.5 °C (60 °F).

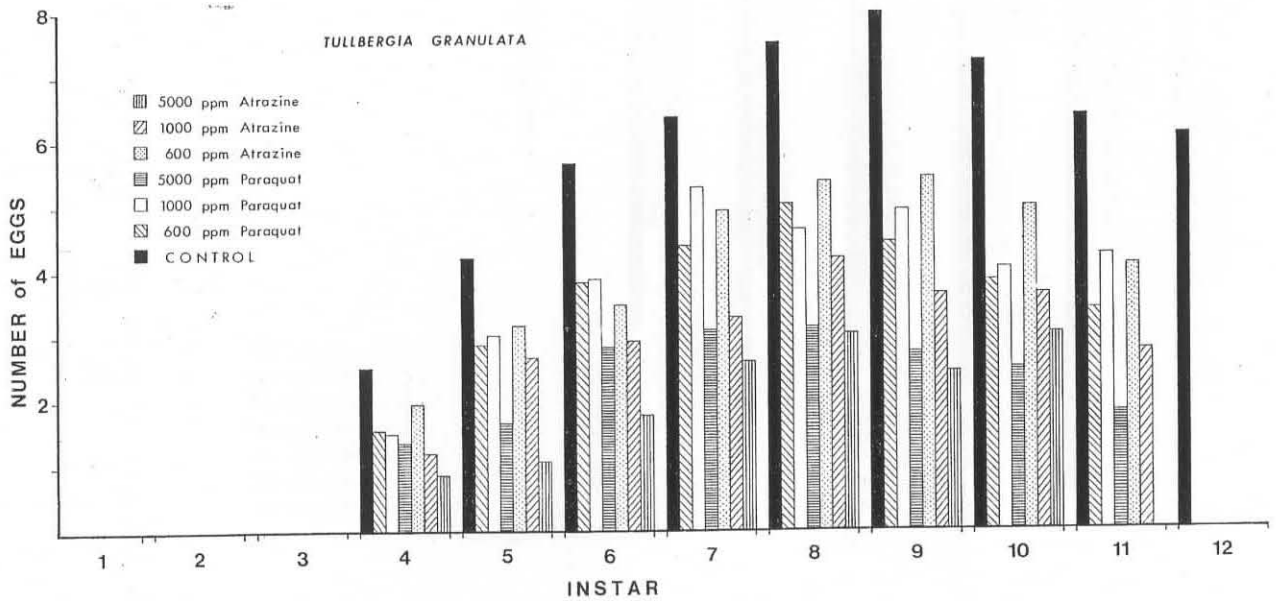

Fig. 4. *Tullbergia granulata*, egg production at 15.5 °C (60 °F).

Figure 3 shows that the treated *F. candida* significantly reduced their egg production at every oviposition as compared to controls. Feeding on atrazine also induced significantly lower egg production than feeding on paraquat (Tukey's t-test, $p \leq .05$). There was no significant difference between 600 ppm and 1,000 ppm of either paraquat or atrazine. At 5,000 ppm of atrazine, only four individuals laid eggs, with less than four eggs at every oviposition.

T. granulata began to lay eggs in the fourth instar, and oviposited in every instar thereafter. As in *F. candida*, oviposition occurred within 24 hours of ecdysis. Eggs were very small, soft and colorless. As they developed, the eggs increased in size and became more opaque. In contrast to *F. candida*, this species laid eggs singly, not in clumps or clusters. Clusters of two to three eggs were found only occasionally. In general, they were dispersed over the substrate. Deposition on the container wall occurred rarely.

T. granulata laid few eggs: one to four at the first oviposition and increased numbers in following ovipositions, until reaching a peak of four to eleven eggs at the eighth to tenth instar. Egg production then declined (Figure 4). All treated *T. granulata* showed significantly reduced egg production as compared to controls. However, there was no difference between paraquat and atrazine treatments. At 5,000 ppm of both herbicides, egg production was significantly different from the lower concentrations (Tukey's t-test, $p \leq .05$). No difference appeared between 600 ppm and 1,000 ppm. Treated *T. granulata* also showed irregularities in oviposition, and some of them did not begin to lay eggs in the fourth instar.

3.4. Egg viability

For determination of egg viability, only those laid on the substrate were counted. They were transferred to fresh jars after the chorion had ruptured, and incubated until eclosion. Eggs laid on the container wall were counted and removed, because these eggs would soon desiccate. Eggs attacked by molds were not included. Some treated *F. candida* were found to lay "incomplete" eggs, analogous to a mass of jelly-like substance, and these were also excluded from viability counts. Viability was calculated as follows:

$$\frac{\text{Total number hatching at one instar}}{\text{Total number of eggs at same instar}} \times 100$$

Results indicate that, in general, there was no difference between controls and treated Collembola. However, egg viability of *F. candida* fed on 1,000 ppm and especially 5,000 ppm of paraquat, by the fifth oviposition, drastically decreased (Table 4). *T. granulata* fed on 5,000 ppm of paraquat showed only slightly lower viability as compared to controls (Table 5). At least in *F. candida*, high concentration of paraquat may affect egg viability. Atrazine at 5,000 ppm, while reducing egg production, did not reduce egg viability.

3.5. Incubation period

A number of eggs laid by animals of the same instar were taken randomly from each treatment concentration as well as from control. These eggs were placed in fresh jars and incubated at 15.5 °C (60 °F). The duration of embryonic development was recorded for each egg. The relative frequency of development times, in percent, are illustrated in Figures 5 and 6. Statistical analysis showed that eggs of Collembola fed on treated yeast needed a longer incubation period than those of control animals (t-test, $p \leq .01$ for *F. candida* and $\leq .05$ for *T. granulata*).

4. Discussion

Observation concerning the life history of *F. candida* given by Marshall & Kevan (1962) and Green (1964) differ somewhat from the present study, probably because rearing methods were different. In many respects, the present observations were conducted under conditions similar to those of Snider (1971) and Snider & Butcher (1973), thus providing a basis for comparing results.

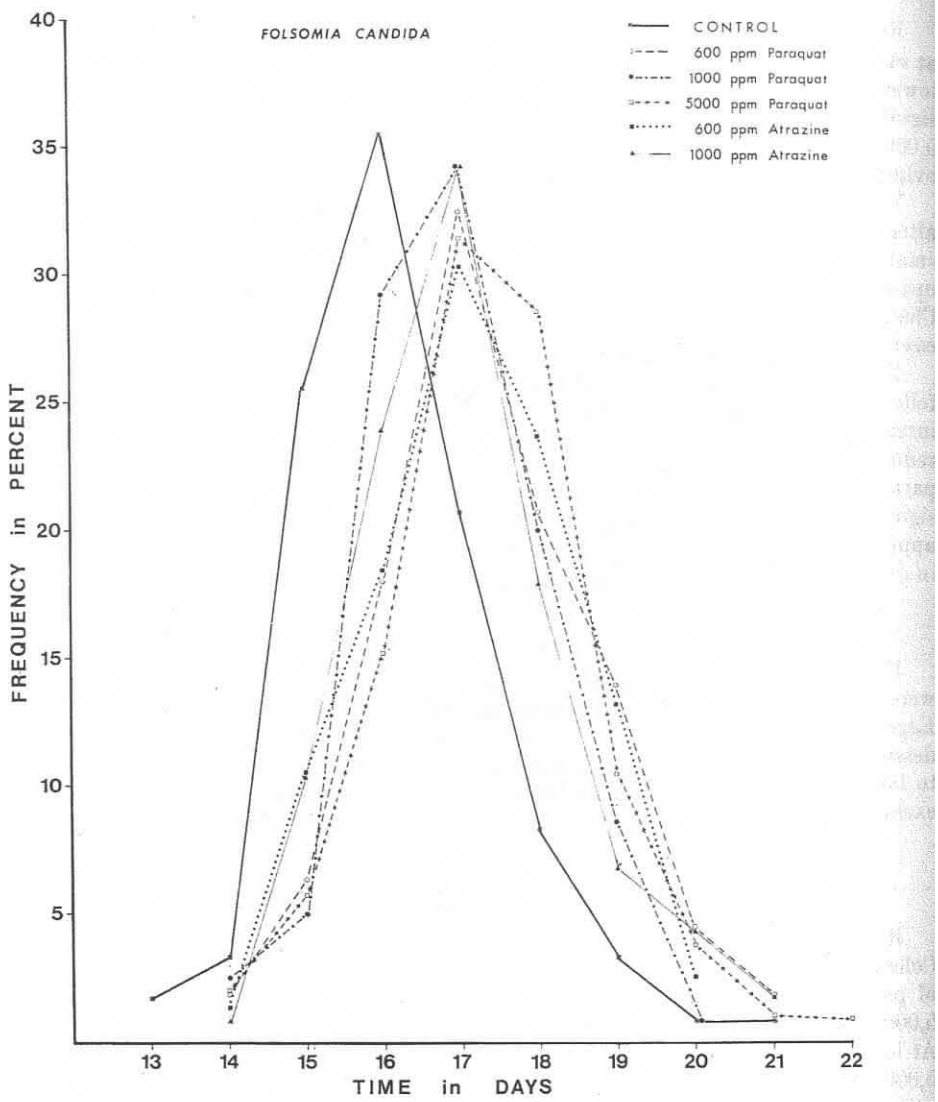

Fig. 5. *Folsomia candida*, incubation period at 15.5 °C (16 °F). (Relation between number of eggs and time needed for hatching.)

Table 4. *Folsomia candida*, egg viability (in percent) per oviposition, at 15.5 °C (60 °F)

Oviposition	Control	Paraquat			Atrazine		
		600 ppm	1,000 ppm	5,000 ppm	600 ppm	1,000 ppm	5,000 ppm
1	96.3	96.4	95.3	93.9	100.0	93.0	—
2	93.3	97.7	97.0	95.0	98.2	98.8	100.0
3	96.5	95.3	95.1	92.1	99.0	88.7	100.0
4	89.7	95.1	75.4	90.9	99.0	89.9	100.0
5	96.8	95.2	73.5	81.3	98.0	78.3	100.0
6	96.1	96.2	72.5	60.7	95.5	91.7	
7	89.1	89.0	85.9	54.6	89.4	90.0	
8	90.3	89.2	89.0				

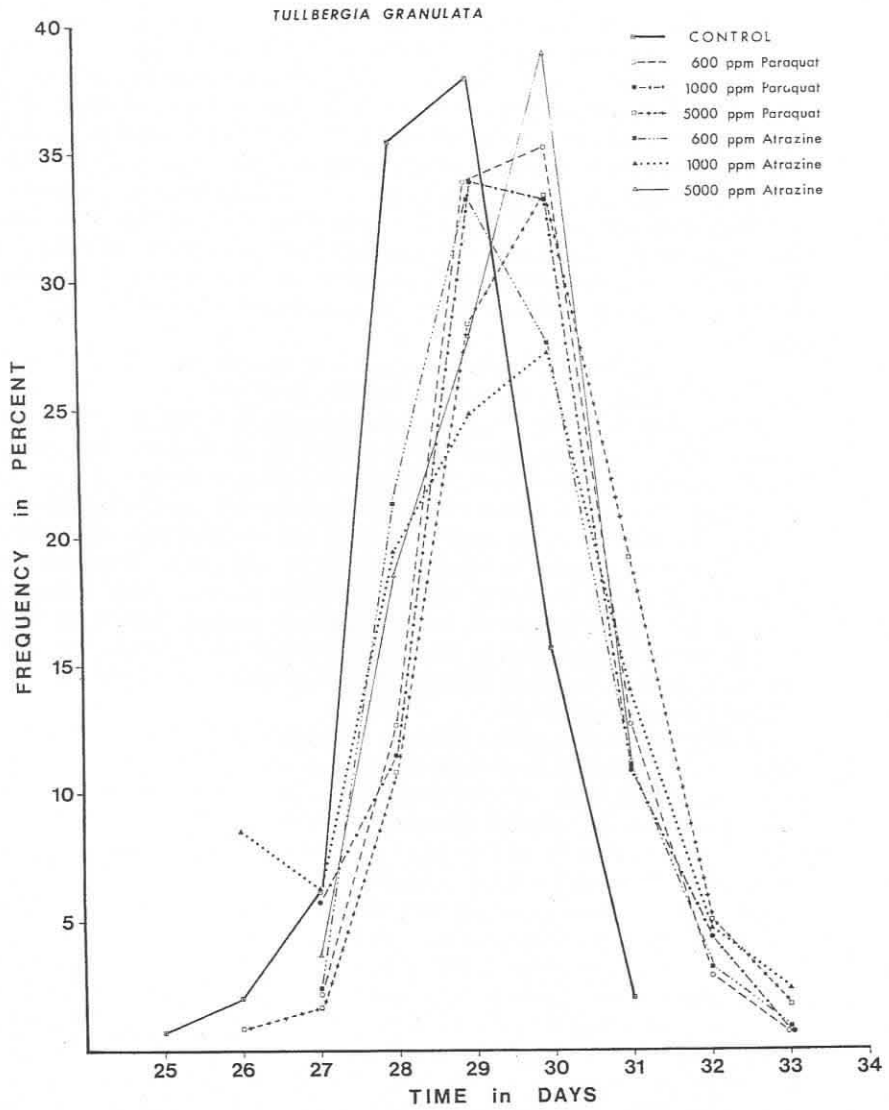

Fig. 6. *Tullbergia granulata*, incubation period at 15.5 °C (60 °F). (Relation between number of eggs and time needed for hatching.)

Table 5. *Tullbergia granulata*, egg viability (in percent) per oviposition, at 15.5 °C (60 °F)

Oviposition	Control	Paraquat			Atrazine		
		600 ppm	1,000 ppm	5,000 ppm	600 ppm	1,000 ppm	5,000 ppm
1	100.0	100.0	100.0	85.2	92.3	95.8	100.0
2	95.2	91.1	95.0	93.9	88.9	94.3	100.0
3	98.2	98.7	97.4	98.3	89.9	93.1	100.0
4	96.6	97.8	94.3	92.1	98.0	96.3	100.0
5	96.4	99.0	95.5	98.8	96.3	95.1	100.0
6	96.8	96.7	95.9	94.4	97.2	95.8	100.0
7	95.6	96.1	96.3	95.9	96.9	95.8	100.0
8	96.7	95.5	95.1	89.7	95.9	94.1	
9	95.9						

Up to the present time, the life history of *T. granulata* has not been reported. Breeding biology and post-embryonic development of *Tullgergia krausbaueri* (BÖRNER) were observed by HALE (1965a, b), and PETERSEN (1971) found parthenogenesis in the species. HALE's records state that *T. krausbaueri* began to lay eggs in the third instar, although only a single specimen was found to do so from among 23 individuals observed. Maximum egg production was reached in the seventh and eighth instars with averages of 7.6 and 7.25 eggs. The present study shows that parthenogenetic *T. granulata* attained sexual maturity in the fourth instar, and maximum egg production in the eighth and ninth instars with means of 7.5 and 7.95 eggs, respectively. These data indicate that there is no great variation in fecundity between the two species. Egg viability of *T. granulata* was high at every oviposition, while PETERSEN (1971) reported low viability due to injuries during transfer of eggs from one chamber to another.

Observations on *T. krausbaueri* and other species have shown that instar duration increases with progressing age (HALE 1965b). In *F. candida*, the first instar was reported to be of longer duration than following immature instars (GREEN 1964; SNIDER 1971; SNIDER & BUTCHER 1973). The present study clearly shows that the first stadium of *T. granulata* lasted longer than subsequent immature and mature instars, at least up to the eleventh. Second and third instars were of shortest duration followed by a gradual increase (Figure 2).

High mortality was observed in animals fed on 5,000 ppm of atrazine. It was not clear whether this was caused by the herbicide or by starvation. Repellent effects have been observed by EIJSACKERS (1975) when *Onychiurus quadriocellatus* GISIN were given a choice between untreated quadrants and quandrants treated with 2,4,5-T. EIJSACKERS (1978) later found that application of 2,4,5-T to food increased mortality. Since 5,000 ppm atrazine appeared to have repellent effects, starvation could have been the cause of mortality in *F. candida* and *T. granulata*.

Modification in fecundity, life span and egg viability in several insects due to sublethal effects of insecticides have been known for several years (KNUTSON 1955; EDWARDS & THOMPSON 1973). Although the mechanism is not totally understood, the reproductive system appears to be affected (MORIARTY 1969). Another explanation, given by DUNCAN (1963), states that reduced feeding could cause reduced fecundity since *Aedes aegypti* LINN. treated with a sublethal dose of dieldrin, did not eat as much as controls. In the present study, reduced feeding occurred only with atrazine, and then only at a dosage of 5,000 ppm. But fecundity was reduced at all dosages of both herbicides. In addition, *F. candida* laid eggs that were "incomplete" and showed low viability and prolonged embryonic development. These results indicate that the reproductive system was indeed affected by both paraquat and atrazine. With *O. quadriocellatus* as test animal, EIJSACKERS (1978a) found that egg production was also curtailed by food treated with 2,4,5-T. However, in contrast to the present study, instar duration of *O. quadriocellatus* was shorter in treated animals than in controls. This discrepancy may be due to the different food sources and herbicides used.

Based on the argument that herbicides are applied to plants, not to the soil, some investigators believed that herbicides have no direct effects on soil animals (FOX 1964; CURRY 1970; EDWARDS & THOMPSON 1973). Only triazine herbicides, including atrazine, have direct effects (EDWARDS 1970). But elimination of weeds could reduce food sources for some species, and indirectly affect soil animal populations. In addition, foliar-applied herbicides can also penetrate soil by rainfall, although concentrations are then considerably lower. The results of the present study indicate that paraquat and atrazine can directly affect Collembola, and thus help explain previous reports. CURRY (1970) found a reduction of Collembola in grasslands after it had been sprayed with paraquat, while FOX (1964) and more recently POPOVICI et al. (1972) reported similar results after application of atrazine. Fecundity, instar duration, hatching time, and mortality are among the most important parameters of population dynamics. Any detriment to those parameters will consequently affect populations. Such effects can be documented in the laboratory, but their magnitude in the field may depend on a species' ability to avoid contaminated food.

5. Summary · Zusammenfassung

The side effects of the herbicides paraquat and atrazine upon *Folsomia candida* (Willem) and *Tullbergia granulata* Mills were studied under laboratory conditions. Concentrations of 600, 1,000 and 5,000 ppm of the compounds were fed to the species starting at first instar. Daily observations were made over 22 weeks. Significantly high mortalities were observed only at 5,000 ppm of atrazine. However, it appeared that repellent properties of atrazine accounted for the mortalities over an extended period of time. Both *F. candida* and *T. granulata* exhibited significantly longer instar duration, especially at 5,000 ppm, than the controls. Oviposition by the species were irregular in the treatments. Egg viability in *T. granulata* may be affected by 5,000 ppm paraquat, while in spite of reduced egg production application of 5,000 ppm atrazine resulted in a high percentage of viability of eggs. Paraquat at 1,000 and 5,000 ppm.

Die Nebenwirkungen der Herbizide Atrazin und Paraquat auf *Folsomia candida* und *Tullbergia granulata* (Collembola)

Der Einfluß der Herbicide Paraquat und Atrazin auf die Lebensfunktionen von *Folsomia candida* (Willem) *Tullbergia granulata* Mills wurde im Labor untersucht. Die Herbicide wurden den Tieren, sobald sie geschlüpft waren, in Konzentrationen von 600, 1000 und 5000 ppm verfüttert; die Beobachtungen wurden täglich während 22 Wochen durchgeführt. Eine signifikante Sterblichkeit ergab sich nur bei Applikation von 5000 ppm Atrazin. Es schien, daß die repellente Wirkung von Atrazin über längere Zeit zur hohen Mortalität beiträgt. Sowohl *F. candida* als auch *T. granulata* zeigten, im Vergleich zu den Kontrollen, eine signifikant verlängerte Dauer der Entwicklungsstadien, speziell bei Applikation von 5 000 ppm. Der Rhythmus der Eiablagen war bei behandelten Tieren unregelmäßig. Die Lebensfähigkeit der Eier von *Tullbergia granulata* mag durch die Applikation von 5000 ppm Paraquat beeinträchtigt sein, doch trotz der eingeschränkten Eiproduktion führte die Applikation von 5000 ppm Atrazin zu einem hohen Prozentsatz lebensfähiger Eier. Die Entwicklungsfähigkeit der Eier von *F. candida* war bei Applikation von 1000 und 5000 ppm Paraquat stark reduziert. Im allgemeinen zeigten die Eier der behandelten Tiere eine verzögerte Embryonalentwicklung.

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7. References

Chase, R. W., & M. F. Meggit, 1976. Weed control. No till corn: 4. Ext. Bull. E-907. Coop. Ext. Serv., M. S. U. Jan. 1976.

Curry, J. P., 1970. The effects of different methods of sward establishment and the effects of herbicide paraquat and dalapon on the soil fauna. Pedobiologia **10**, 329—361.

Duncan, J., 1963. Post-treatment effects of sublethal doses of dieldrin on the mosquito *Aedes aegypti* L. Ann. Appl. Biol. **52**, 1—6.

Edwards, C. A., 1970. Effects of herbicides on the soil fauna. Proc. 10th Brit. Weed Control Conf.: 1052—1062.

— A. E. Whitting, 1972. Paraquat and slit-seeding. Rothamsted Exp. Sta. Report for 1971: 212 to 213.

— A. R. Thompson, 1973. Pesticides and the soil fauna. Residue Review **45**, 1—79.

Eijsackers, H., 1975. Effects of herbicide 2,4,5-T on *Onychiurus quadriocellatus* Gisin (Coll.). In: Jan Vaněk (ed.): Progress in Soil Zoology. Academia, Publishing House of the Czechoslovak Acad. of Sci. Prague: 481—488.

— 1978a. Side effects of the herbicide 2,4,5-T on reproduction, food consumption, and moulting of springtail *Onychiurus quadriocellatus* Gisin (Collembola). Z. Ang. Ent. **85**, 341—360.

— 1978b. Side effects of the herbicide 2,4,5-T affecting mobility and mortality of springtail *Onychiurus quadriocellatus* Gisin (Collembola). Z. Ang. Ent. **86**, 349—372.

Fox, C. J. B., 1964. The effects of five herbicides on the numbers of certain invertebrate animals in grassland soil. Can. J. Plant Sci. **44**, 405—409.

Green, C. D., 1964. The life history and fecundity of *Folsomia candida* (Willem) var. *distinta* (Bagnall) (Collembola: Isotomidae). Proc. R. Ent. Soc. London (A) **39**, 125—128.

Hale, W. G., 1965a. Observation of the breeding biology of Collembola (II). Pedobiologia **5**, 161 to 177.

— 1965b. Post-embryonic development in some species of Collembola. Pedobiologia **5**, 228—243.

Knutson, H., 1955. Modification in fecundity and life span of *Drosophila melanogaster* Meigen following sublethal exposure to an insecticide. Ann. Ent. Soc. Amer. **48**, 35—39.

MARSHALL, V. G., & D. K. McE. KEVAN, 1962. Preliminary observations on the biology of *Folsomia candida* WILLEM, 1902 (Collembola: Isotomidae). Can. Entomol. **94**, 515—586.
MORIARTY, F., 1969. The sublethal effects of synthetic insecticides on insects. Biol. Rev. **44**, 321—357.
NELSON, L. V., L. S. ROBERTSON, M. H. ERDMAN, R. G. WHITE & D. QUISENBERRY, 1976. Guide lines. No till corn: 1. Ext. Bull. E-904. Coop. Ext. Serv., M. S. U. March 1976.
PETERSEN, H., 1971. Parthenogenesis in two common species of Collembola: *Tullbergia krausbaueri* (BÖRNER) and *Isotoma notabilis* SCHÄFFER. Rev. Ecol. Biol. Sol. **8**, 133—138.
POPOVICI, I., G. STAN, V. STEFAN, R. TOMESCU, A. DUMEN, A. TARTA & F. DAN, 1977. The influence of atrazine on soil fauna. Pedobiologia **17**, 209—215.
SNIDER, R. J., J. SHADDY & J. W. BUTCHER, 1969. Some laboratory techniques for rearing soil arthropods. Mich. Ent. **1**, 357—362.
SNIDER, R. M., 1971. Laboratory observations on the biology of *Folsomia candida* (WILLEM) (Collembola: Isotomidae). Rev. Ecol. Biol. Sol. **10**, 103—124.
— J. W. BUTCHER, 1973. The life history of *Folsomia candida* (WILLEM) (Collembola: Isotomidae) relative to temperature. The Great Lakes Ent. **6**, 97—106.
TUKEY, J. W., 1953. The problem of multiple comparisons. Mimeographed notes. Princeton, N. J.: Princeton University Math. Dept.

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